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# Cotton genotypic variation in the photosynthetic response to irradiance

W.T. PETTIGREW

*USDA-ARS Crop Genetics and Production, P.O. Box 345, Stoneville, MS 38776, USA*

## Abstract

The photosynthetic response of 8 cotton (*Gossypium hirsutum* L.) genotypes to changing irradiance was investigated under field conditions during the 1998 through 2000 growing seasons. Equations developed to describe the response of net photosynthetic rate ($P_N$) to photosynthetic photon flux density (PPFD) demonstrated that, across all irradiances, the two okra leaf-type genotypes photosynthesized at a greater rate per unit leaf area than all of the six normal leaf-type genotypes. This superior photosynthetic performance of the okra leaf-type genotypes can be partially explained by their 13 % greater leaf chlorophyll content relative to that of the normal leaf-type genotypes. The 37 % reduction in leaf size brought upon by the okra leaf trait may have concentrated the amount of photosynthetic machinery per unit leaf area. Nevertheless, the lack of sufficient canopy leaf surface area suppressed the potential yield development that could accompany the higher $P_N$ per unit leaf area.

*Additional key words*: area leaf mass; chlorophyll; dark respiration rate; *Gossypium*; leaf area; protein.

## Introduction

The photosynthetic process is an integral part of plant growth and development, with the carbon assimilates necessary for yield production ultimately produced *via* photosynthesis. Thus anything substantially altering the net rate of photosynthesis ($P_N$) has potential to also alter growth. Because photon energy is one of the principle components driving the photosynthetic process, fluctuations in the irradiance can cause substantial alterations in the photosynthetic rate observed.

The lint yield of cotton (*Gossypium hirsutum* L.) grown in the mid-southern region of the US cotton production belt is limited by the amount of sunlight intercepted during the growing season (Pettigrew 1994). This irradiance limitation to lint yield is probably a C assimilate limitation in lieu of the yield increases produced through $CO_2$ enrichment (Krizek 1986) and the positive association between $P_N$ during the boll filling period and lint yield (Pettigrew and Meredith 1994). The photosynthetic irradiance response for cotton produces an asymptotic model-like curve (Patterson *et al*. 1977, Sassenrath-Cole *et al*. 1996), typical of other $C_3$ plants. The cotton $P_N$-irradiance responses in the both aforementioned studies were developed after the leaves had acclimated to the various photosynthetic photon flux density (PPFD) for a period of approximate 30 min prior to $P_N$ measuement. These responses would be appropriate when exposure to a particular irradiance is prolonged in nature. A slightly different response might be forthcoming when a leaf encounters a rapidly changing irradiance such as occurs with partly cloudy sky conditions, typical of the growing season weather in the humid, temperate mid-southern US. Bauer *et al.* (1997) reported an instantaneous reduction in $P_N$, but a slower and less drastic reduction in stomatal conductance after brief exposure to shade. These gas exchange measurements recovered to pre-shade values after about 5 min.

While genotypic differences have been detected in cotton leaf photosynthesis (Pettigrew *et al.* 1993, Pettigrew and Meredith 1994, Faver *et al.* 1996) and in the photosynthetic response to changing $CO_2$ concentrations (Pettigrew and Turley 1998), little evidence exists documenting differences among cotton genotypes in the photosynthetic response to changing PPFD. Therefore, the objective of this research was to determine if differences in the photosynthetic response to different PPFD existed among a diverse group of cotton genotypes.

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Fax: +662 686 5218; e-mail: pettigrew@ars.usda.gov
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## Materials and methods

Eight cotton genotypes (DPL 20, DPL 20B, FiberMax 819, MD51ne normal, MD51ne okra, PM H1220, PM 1220BR, and STV. 474) were grown in a Bosket fine sandy loam (fine-loamy, mixed, thermic Mollic Hapludalf) field near Stoneville, MS, USA from 1998 through 2000. The genotypes represent a diverse group of maturities and breeding programs, with FiberMax 819 and MD51ne okra possessing the okra leaf-type trait. Plots, consisting of 4 rows 7.62 m long with a 1 m row spacing, were planted in late April of each year in a randomized complete block experimental design with 5 replicates.

$P_N$ were measured on the youngest fully expanded, disease-free, fully sunlit leaves in each plot using a *LI-6200* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA). All measurements were taken using a 1 000 $cm^3$ leaf chamber between 09:00 and 12:00 CDT. For the PPFD-saturated measurements, individual leaves were oriented perpendicular to the sun with PPFD reaching the leaf surfaces ≥1 600 during the measurements. Immediately following the PPFD saturated measurement, a series of 3 shaded PPFD measurements were performed on the same leaf. The first shaded PPFD measurement utilized one layer of shade cloth (= 30 % shade) to partially block photons from reaching the leaf surface. The second shade measurement (= 50 % shade) utilized 2 layers of shade cloth, with the second layer of cloth rotated 45° relative to the first shade cloth layer. The third shade measurement involved covering the leaf chamber with a dark cloth that prevented any sunlight from reaching the leaf surface. Following the final shade measurements, leaves were collected on ice and transported to the laboratory for later determinations of leaf area and contents of chlorophyll (Chl) and soluble proteins.

In the laboratory, leaf area was determined by passing the leaves through a *LI-3100* leaf area meter (*LI-COR*, Lincoln, NE, USA). Then two 0.4-$cm^2$ leaf disks were cut from the leaf for Chl measurement and two were cut for soluble protein determination. The remaining leaf was then dried at 65 °C for at least 48 h, following which leaf dry mass was recorded. Area leaf mass (ALM) was calculated from the leaf area and leaf dry mass measurements.

Chl was extracted from the leaf disks with 5 $cm^3$ of 95 % ethanol over a 24 h period of darkness as described in Pettigrew and Meredith (1994). The Chl concentration of the extract was quantified spectrophotometrically as described by Holden (1976). Soluble protein was extracted by grinding the two leaf disks in 1 $cm^3$ of a grinding buffer containing 0.1 M *Tris* (pH 7.6, HCl), 0.1 M $Na_2B_4O_7$, 5 mM $MgCl_2$, 10 mM ascorbate, 0.1 % (m/v) β-mercaptoethanol, 1 % (m/v) polyvinylpyrrolidone, and 2 M urea. The resulting brei was then centrifuged at 5 000×g for 10 min. The soluble protein in the resulting supernatant was quantified by the method of Bradford (1976).

Statistical analyses of the data were performed by analysis of variance. Means were separated using protected LSD at the $p \leq 0.05$ level. For the $P_N$-PPFD measurements, the four PPFD measurements could be considered sub-plots to the genotype main unit effect. By taking the natural log of both $P_N$ and PPFD and considering PPFD as a continuous effect, a log–log linear trend was fit for $P_N$ as a function of PPFD for each genotype. Converting the log transformed $P_N$ and PPFD values back to the original units resulted in an exponential equation of the form:

$$Y = \alpha X^{\beta}$$

where Y is $P_N$, X is PPFD, α is the antilog of the intercept, and β is the slope. Analysis of variance was utilized to determine whether the intercepts or slopes of the curves differed among the genotypes or years.

## Results and discussion

The $P_N$-PPFD response was determined for eight cotton genotypes during the years 1998–2000. From these values, predictive equations were developed to describe the response of $P_N$ to varying PPFD among the genotypes and years. The $P_N$-PPFD response curves generated for MD51ne okra by the predictive equation in 1998, typical of other genotypes and years, provided close fit to the actual data (Fig. 1*A*). A large F value (4563) demonstrated that the slope was statistically different from zero ($p$>F = 0.001). Statistical analyses indicated that while the slopes did not vary among the genotypes and genotypes did not interact with years, strong year to year variations in the slopes were detected. Slope values ranged from 0.2743 in 1998, 0.2573 in 1999, to 0.2774 in 2000. Although genotypes did not significantly affect equation slopes, the curve intercepts did differ among genotypes (Table 1), but not years. These differences among genotypes for the intercepts and among years for slopes produced different $P_N$-PPFD response curves for each genotype for each year (Fig. 1*B*).

The predicted $P_N$ at various PPFD for the two okra leaf-type genotypes, FiberMax 819 and MD51ne okra, was always greater than that predicted for the normal leaf-type genotypes (Fig. 1*B* and Table 1). The $P_N$ of okra leaf-type lines did not differ statistically from each other and the normal leaf-type lines did not differ among themselves for $P_N$. This difference in $P_N$ between the okra leaf-type and normal leaf-type lines was exhibited with dark respiration rate ($R_D$) at 0 μmol(photon) $m^{-2}$ $s^{-1}$ PPFD (both measured and predicted) and became exponentially greater as the PPFD increased. With the exception of the year 2000 when no genotypic differences were detected

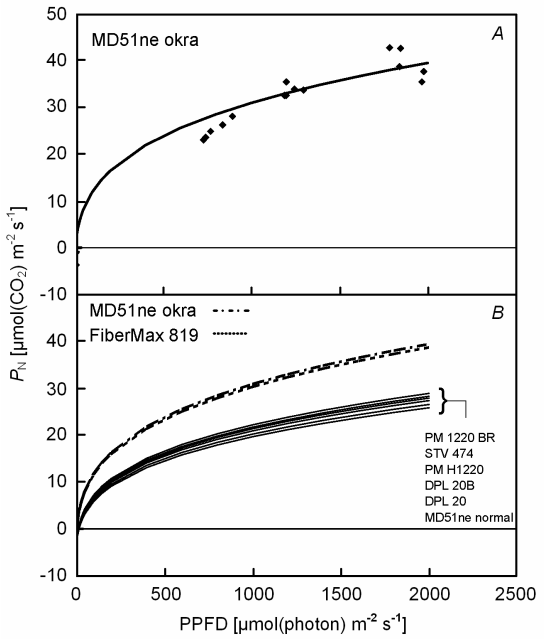


Fig. 1. Photosynthetic response of (*A*) genotype MD51ne okra and (*B*) eight other cotton genotypes to varying photosynthetic photon flux density (PPFD) in 1998. The predictive curves were developed as shown in Materials and methods. The 1998 curves are representative also of those for 1999 and 2000.

in measured $R_D$, genotypic differences in measured $R_D$ closely matched the predicted $P_N$ at 0 μmol(photon) $m^{-2}$ $s^{-1}$ PPFD. However, the predicted values were generally higher than the measured values.

Genotypic differences were detected in many of the leaf physiological traits measured (Table 2). The okra leaf trait reduced the average leaf area by 37 % compared to the normal leaf-type lines, and MD51ne okra was 24 % smaller than the FiberMax 819 okra leaf. Along with smaller leaf area, the okra leaf-type trait elevated the leaf Chl content by 13 % compared with that of the normal leaf-type lines. Similar to the leaf area data, MD51ne okra had an 8 % higher leaf Chl content than FiberMax 819. The okra leaf-type lines in this study tended to have lower ALM than the normal leaf-type lines, in contrast to previous research that found okra leaf-type lines to have higher ALM (Pettigrew *et al.* 1993). This discrepancy was probably due to the fact that the okra leaf-type trait was being expressed in different genetic backgrounds and because different normal leaf-type genotypes were utilized. No genotypic differences were detected in leaf soluble protein content or in the leaf Chl *a:b* ratio.

Okra leaf-type genotypes in this study photosynthesized at higher rates under PPFD saturation than the normal leaf-type genotypes, similar to previous research (Pettigrew *et al.* 1993). Prior to this research, it was

Table 1. Dark respiration rate ($R_D$) and the predicted net photosynthetic rate ($P_N$) at various photosynthetic photon flux densities (PPFD) for eight cotton genotypes during the years 1998–2000. Y axis intercepts equate to the zero PPFD-predicted $P_N$ values. Values within the same column and year followed by the same letter do not differ significantly ($p \leq 0.05$).

| Year | Genotype | Leaf type | $R_D$ | Predicted $P_N$ [μmol($CO_2$) $m^{-2}$ $s^{-1}$] Specific PPFD [μmol(photon) $m^{-2}$ $s^{-1}$] | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0 | 600 | 1 000 | 1 600 |
| 1998 | DPL 20 | normal | -5.17 b | -5.45 b | 16.33 b | 20.29 b | 24.45 b |
| | DPL 20B | normal | -5.28 b | -5.34 b | 16.93 b | 20.98 b | 25.24 b |
| | FiberMax 819 | okra | -1.59 a | -3.93 a | 25.13 a | 30.41 a | 35.96 a |
| | MD51ne normal | normal | -5.73 b | -5.53 b | 15.83 b | 19.71 b | 23.79 b |
| | MD51ne okra | okra | -1.22 a | -3.84 a | 25.65 a | 31.00 a | 36.64 a |
| | PM H1220 | normal | -5.05 b | -5.15 b | 18.05 b | 22.26 b | 26.69 b |
| | PM 1220BR | normal | -4.85 b | -5.28 b | 17.33 b | 21.43 b | 25.76 b |
| | STV. 474 | normal | -5.05 b | -5.23 b | 17.61 b | 21.75 b | 26.12 b |
| 1999 | DPL 20 | normal | -4.87 b | -5.45 b | 13.62 b | 16.93 b | 20.39 b |
| | DPL 20B | normal | -4.27 b | -5.34 b | 14.16 b | 17.55 b | 21.08 b |
| | FiberMax 819 | okra | -2.20 a | -3.93 a | 21.51 a | 25.93 a | 30.54 a |
| | MD51ne normal | normal | -4.87 b | -5.53 b | 13.17 b | 16.41 b | 19.81 b |
| | MD51ne okra | okra | -1.98 a | -3.84 a | 21.98 a | 26.46 a | 31.14 a |
| | PM H1220 | normal | -4.74 b | -5.15 b | 15.16 b | 18.68 b | 22.37 b |
| | PM 1220BR | normal | -4.93 b | -5.28 b | 14.51 b | 17.95 b | 21.54 b |
| | STV. 474 | normal | -5.17 b | -5.23 b | 14.76 b | 18.23 b | 21.86 b |
| 2000 | DPL 20 | normal | -5.11 a | -5.45 b | 16.86 b | 20.94 b | 25.25 b |
| | DPL 20B | normal | -5.42 a | -5.34 b | 17.47 b | 21.65 b | 26.05 b |
| | FiberMax 819 | okra | -3.39 a | -3.93 a | 25.83 a | 31.28 a | 37.02 a |
| | MD51ne normal | normal | -1.37 a | -5.53 b | 16.34 b | 20.35 b | 24.57 b |
| | MD51ne okra | okra | -3.74 a | -3.84 a | 26.36 a | 31.89 a | 37.72 a |
| | PM H1220 | normal | -4.29 a | -5.15 b | 18.61 b | 22.96 b | 27.54 b |
| | PM 1220BR | normal | -5.21 a | -5.28 b | 17.88 b | 22.11 b | 26.58 b |
| | STV. 474 | normal | -4.11 a | -5.23 b | 18.16 b | 22.44 b | 26.95 b |

unclear whether this superior photosynthetic performance for the okra leaf-type lines was also manifest at lower, non-saturating PPFD. Under non-saturating conditions, it was possible that the photosynthetic advantage enjoyed by the okra leaf-type lines could be overshadowed by a dominating PPFD limitation to maximal $P_N$. Thus the okra leaf-type lines were able to maintain their superior photosynthetic performance per unit leaf area relative to the normal leaf-type genotypes even when the solar radiation dipped to a point that irradiance became a prominent rate-limiting factor to photosynthesis.

One of the traits that allowed the okra leaf-types to photosynthesize at a higher rate for any given irradiance was their greater leaf Chl content relative to the normal leaf-type lines. The higher leaf Chl content in okra leaf-type line is similar to that previously reported for okra–normal leaf-type comparisons (Pettigrew *et al.* 1993). In this prior research, thicker leaves for the okra-leaf-type line were associated with greater ALM, leaf Chl content, and leaf soluble protein content, indicative of an increased content of the photosynthetic apparatus per unit leaf area. The increased leaf Chl content for MD51ne okra and FiberMax 819 provides support for the idea that these genotypes have more photosynthetic components per unit leaf area than the normal leaf-types. Nevertheless, the lack of an increased ALM and leaf soluble protein content in the current study does not support that premise. Nonetheless, okra leaf-type genotypes have higher $P_N$ and leaf Chl content per unit leaf area than their normal leaf-type counterparts.

Table 2. Cotton leaf chlorophyll (Chl) content [mg $m^{-2}$], Chl *a:b* ratio, soluble protein content [g $m^{-2}$], leaf area [$cm^2$], and area leaf mass, ALM [g $m^{-2}$] for eight cotton genotypes averaged across the years 1998–2000. Values within the same column and followed by the same letter do not differ significantly ($p \leq 0.05$).

| Genotype | Leaf-type | Chl | Chl *a:b* | Protein | Leaf area | ALM |
|---|---|---|---|---|---|---|
| DPL 20 | normal | 360 c | 3.86 a | 11.8 a | 124 ab | 71.0 ab |
| DPL 20B | normal | 368 c | 3.80 a | 12.2 a | 125 a | 73.4 a |
| FiberMax 819 | okra | 412 b | 3.64 a | 12.7 a | 87 c | 66.7 c |
| MD51ne normal | normal | 387 bc | 3.65 a | 11.8 a | 118 ab | 68.2 bc |
| MD51ne okra | okra | 443 a | 3.80 a | 11.2 a | 66 d | 65.8 c |
| PM H1220 | normal | 363 c | 3.88 a | 12.0 a | 124 ab | 68.7 bc |
| PM 1220BR | normal | 386 bc | 3.88 a | 12.4 a | 124 ab | 68.2 bc |
| Stv. 474 | normal | 403 b | 3.84 a | 11.5 a | 115 b | 70.5 ab |

The PPFD response curves in this research were produced without an acclimation period to the different irradiances customary in previous cotton photosynthesis-PPFD response research (Patterson *et al.* 1977, Sassenrath-Cole *et al.* 1996). While these instantaneous measurements may not reflect an equilibrium obtained after prolonged exposure to a given PPFD, they do reflect the performance expected under the rapidly changing sky conditions common during the growing season to the humid southeastern USA.

In conclusion, the okra leaf-type cotton genotypes photosynthesized at a greater rate than any of the normal leaf-type genotypes utilized in this study. These genotypic differences were consistent across years, and remained intact even when the irradiance was low enough that it became the predominant factor limiting photosynthesis. Nonetheless, the reduction in overall leaf area index and therefore canopy solar radiation interception (Heitholt *et al.* 1992) prevent these high photosynthesizing okra leaf-type cottons from having superior yield to their normal leaf-type counterparts.

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